BIKEN JOURNAL Vol. 26 63–65, 1983

SHORT COMMUNICATION

IMPROVED METHOD FOR SEPARATION ON CM-CELLULOSE OF THE TRYPOMASTIGOTE FORM OF *TRYPANOSOMA CRUZI* FROM FORMS GROWN IN FIBROBLAST CELL CULTURES

HIROJI KANBARA and TOSHIO NAKABAYASHI

Department of Protozoology, Research Institute for Microbial Diseases, Osaka University, Yamadaoka 3–1, Suita, Osaka 565, Japan

(Received November 6, 1982)

Trypomastigotes grown in fibroblast cell (L-cell) culture were more effectively and more rapidly separated from other cells on a CM-cellulose column with culture medium (MEM with 10% calf serum) for elution instead of phosphate-saline-glucose buffer (PSG). This effective separation was shown to be due to the presence of serum. Trypomastigotes weakly adhered to CM-cellulose resin by the tip of the body (mainly the flagellar tip) when they were suspended with CM-cellulose resin in PSG. Serum seemed to disturb the adhesion of trypomastigotes to the resin, but not the adhesion of amastigotes and fibroblast cells. Therefore, only trypomastigotes were rapidly eluted from a CM-cellulose column in the presence of serum.

In 1977 we reported the separation on CM-cellulose of the trypomastigote form of *Trypanosoma cruzi* from forms grown in fibroblast cell culture (Kanbara et al. 1977). However, only 20–30% of the trypomastigotes were recovered, and they took a long time to collect because they passed through the column gradually. In the present work, we found that when culture medium was used for elution instead of phosphate-saline-glucose buffer (PSG), more than 90% of the trypomastigotes were rapidly recovered.

For collection of forms grown in fibroblast cell cultures, blood form trypomastigotes were isolated from mice infected with the Tulahuen strain of *T. cruzi* by density centrifugation on a Ficoll-Conray column (specific gravity 1.094) and inoculated into a fibroblast cell (L-cell) culture. The culture was maintained at 37 C in a 5%-CO_2 incubator, changing the medium (MEM with 10% calf serum) every three days and adding new fibroblast cells occasionally. After 2 to 3 months, overlay medium containing trypomastigotes, mainly aggregated amastigotes and fibroblasts released from the culture plate were collected and centrifuged at 150 *g* for 5 min to remove roughly aggregated amastigotes and fibroblasts. The supernate was recentrifuged at 1,300 *g* for 10 min and the resulting supernate was collected for later use,

while the precipitate was dispersed in a suitable volume of supernate to give a trypomastigote density of about 3×10^7/ml.

Fresh CM-cellulose powder (Serva) was suspended in distilled water, stood for at least 2 h and then placed in a glass syringe with a wad of glass wool at the bottom to form a CM-cellulose column (2.5×5 cm). The column was equilibrated with PSG, pH 7.2, prepared from 300 ml of 0.2 *M* phosphate buffer (pH 7.2), 300 ml of 0.85% (wt/vol) NaCl and 400 ml of 2.5% (wt/vol) glucose (Lanham 1968). Then 30 ml of trypomastigote mixture was layered on the top of the column and the supernatant medium was applied after the sample had entered the column. The elution rate was adjusted to 6 to 8 ml/min by changing the position of the exit tube. As trypomastigotes came out almost simultaneously with the medium, this fraction of eluate was easily recognized by its orange color due to phenol red. After the sample reached the exit, 50 ml of eluate was collected in a centrifuge tube. In this way, more than 90% of the trypomastigotes were recovered at nearly their initial concentration free from contamination with other cells. Amastigotes and fibroblasts remained adhering to the CM-cellulose resin in these conditions and thus pure trypomastigotes were eluted.

To determine why trypomastigotes easily pass through a CM-cellulose column, the effects of MEM and serum were examined. The precipitate of trypomastigotes was dispersed in MEM, MEM containing 10% calf serum, PSG (pH 7.2) and PSG containing 10% calf serum, and applied to a CM-cellulose column and the same solution was used for elution. Recovery of trypomastigotes was good only when serum was present in the solution. This result suggested that trypomastigotes grown in fibroblast cell culture adhered to the adsorbent weakly in PSG (pH 7.2) and MEM and thus passed through the column only gradually, but that serum disturbed their adhesion to the adsorbent and so caused them to be eluted from the column rapidly. When the elution fluid was changed from PSG to MEM containing 10% calf serum during the elution, trypomastigotes were rapidly eluted but their recovery was less than when MEM containing 10% calf was used from the beginning. For further study of this phenomenon, trypomastigotes from cell culture were washed with PSG (pH 7.2) and mixed with CM-cellulose resin or DEAE-cellulose resin that had been equilibrated with PSG (pH 7.2), and kept for 3 min at room temperature. Then, some of them were promptly examined under a light microscope and others were after being stained with Giemsa. Only a few trypomastigotes were seen adhering to CM-cellulose resin by one tip of the body, but many trypomastigotes were seen adhering tightly to DEAE-cellulose resin by various parts of the body. Amastigotes adhered tightly to both resins. These observations explain why trypomastigotes were eluted gradually from CM-cellulose when PSG was used for elution and rapidly when medium was used. When MEM medium with 10% calf serum was used, trypomastigotes were eluted gradually from a DEAE-cellulose column. This result is consistent with the findings of Souza et al. (1977) and Nogueira et al. (1981) that blood form trypomastigotes have a highly negative surface charge.

The present method can also be used to separate blood form trypomastigotes from white cells after removal of red cells by density centrifugation on a Ficoll-Conray column.

REFERENCES

Kanbara, H., Fukuma, T., Nakabayashi, T. 1977. Separation with CM-cellulose of the trypomastigote form of *Trypanosoma cruzi* from the forms grown in fibroblast cell cultures. Biken J. 20: 147–149.

Lanham, S. M., 1968. Separation of trypanosomes

from the blood of infected rats and mice by anion exchangers. Nature 218: 1273–1274.

Nogueira, N., Chaplan, S., Tydings, J. D., Unkeless, J., Cohn, Z. 1981. *Trypanasoma cruzi*: Surface antigens of blood and culture forms. J. Exp. Med. 153: 629–639.

Souza, W., Arguello, C., Martinez-Pallomo, A., Trissl, D., Gonzalez-Robles, A., Chiari, E. 1977. Surface charge of *Trypanosoma cruzi*. Binding of cationized ferritin and measurement of cellular electrophoretic mobility. J. Protozool. 24: 411–415.